Remarks

Applicants herewith submit this Supplemental Amendment and Reply to the Office action dated August 3, 2009. The Amendment and Reply, originally filed on January 4, 2010 with the Request for Continued Examination and Request For Extension of Time, and a payment of Request for Continued Examination and one-month late fees, inadvertently omitted Exhibits A and B with the submitted Reply. For the Examiner's convenience, Applicants are hereby re-submitting the Amendment and Reply which includes Exhibits A and B. No other changes are introduced into the Reply. No additional fees are believed to be due. Applicants respectfully request that the Examiner consider this Supplemental Amendment and Reply along with Exhibits A and B when considering Applicants response to the Office Action.

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 2, 4-10, 13, 16-18, 20, 21, 23, 25-27, 29-42, 44-56, 60, and 61 are pending in the application, with claim 1 being the independent claim. Claims 29-31, and 35-37 are withdrawn from prosecution. As process claims which depend from and include all the limitations of the product claims currently under examination, Applicants reserve the right to request rejoinder and examination of the withdrawn claims should the product claims be found allowable. Claims 28, 43, and 57-59 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Applicants reserve the right to pursue the subject matter of all earlier pending claims in related applications. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Claims 1, 5, 13, 17, 23, 27, 34, 44, 45, 50, 53, 54, 55, 56, and 61 have been amended. Support for the amendment to claim 1 adding the limitation "wherein said complex adopts a micro-particle structure in the form of a cage-like matrix" can be found in the specification at paragraph [0133], and also in International Patent Application No. PCT/DK02/002299, which corresponds to the U.S. Patent Application No. 10/114,957 (Publication No. US 2003/0118635 A1), allowed on December 4, 2009 (hereinafter "Dalsgaard") on page 22, lines 21-24, which is incorporated into the instant application by reference in paragraph [0126]. Additional amendments to claim 1, as well as amendments to claims 50, 53, 54, and 55 merely clarify the language of the claims, and are not believed to alter the scope or subject matter of the claims in any way. Amendments are sought to claims 13, 44, 45, and 61 to correct dependencies and to maintain proper antecedent basis. Amendments are sought to claims 5, 17, and 27 to correct minor grammatical errors. Support for the amendment to claim 23 can be found in the original claim 23, as filed. Support for the amendment to claim 34 can be found in the original claim 32, as filed. Support for the amendment to claim 56 can be found in the specification at paragraph [0013]. Accordingly, no new matter has been added by these amendments.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections Under 35 U.S.C. § 102(b)

The Examiner rejected claims 1, 2, 4, 5, 9, 10, 13, 16-18, 20, 21, 23, 25-27, 32, 34, 38-43, 46, 47, 49-55, and 57-61 under 35 U.S.C. § 102(b) as allegedly being

anticipated by Foldvari *et al.* (WO 99/11247, hereinafter "Foldvari") (Office Action, hereinafter "OA," at page 3). Applicants respectfully traverse this rejection.

Specifically, the rejected claims all recite a construct for transdermal delivery of an immunogen comprising at least one cationic sterol. The Examiner argues that Foldvari discloses sterols such as cholesterol, coprostanol, cholestanol, and cholestane. Furthermore, the Examiner states that "absent evidence to the contrary, the sterol of Foldvari is necessarily a cationic sterol." (OA at pager 4). Applicants respectfully disagree. The term "cationic sterol" is defined in Dalsgaard on page 27, lines 8-9 as "a sterol carrying a net ***positive*** charge at pH 7.0" (emphasis added). Cholesterol, coprostanol, cholestanol, and cholestane are steroid precursors or metabolites, all of which carry a net ***neutral*** charge at pH 7.0. To illustrate this point, Applicants submit a copy of pages from the Merck index (Exhibit A) describing compounds (5α)-Cholestane (2199), Cholestanol (2200), Cholesterol (2201), and Coprosterol (also known as choprostanol) (2524). Applicants respectfully draw the Examiner's attention to the fact that none of these compounds have groups that are ionizable at pH 7.0. The claim is anticipated by a reference only if the reference teaches each and every element of the claim. (MPEP § 2131). Foldvari does not disclose a construct for transdermal delivery comprising ***cationic*** sterols, and thus does not anticipate any of the claims 1, 2, 4, 5, 9, 10, 13, 16, 18, 20, 21, 23, 25-27, 32, 34, 38-43, 46, 47, 49-55, 57, 60, and 61 .

Furthermore, in an effort to advance prosecution, and not in acquiescence to any pending rejections, Applicants have amended claim 1 to recite that the immunogen delivery system forms a complex which adopts a micro-particle structure in the form of a cage-like matrix. Support for this amendment is found at paragraph [0133] of the

specification as filed, under the section entitled "Immunogen Delivery System." In addition, Dalsgaard, a U.S. patent application publication which has been incorporated by reference in its entirety into the present specification, specifies that the complexes of the present invention may adopt a microparticular structure in the form of a "***cage-like*** matrix similar to that known as an immune stimulating complex (Iscom)." (*See* p. 4, ll. 1-3 of Dalsgaard, emphasis added). The term "Iscom structure" is further defined in Dalsgaard as "***[r]igid cage-like*** matrix characterized by an icosahedral symmetry" on page 2, ll. 21-24 (emphasis added).

In contrast, Foldvari discloses a composition of flexible lipid vesicles (liposomes) for transdermal administration of immunogen, wherein a lipid vesicle is composed of a series of ***lipid bilayers***. Foldvari at p. 6, ll. 1-7 and Figure 1, emphasis added. Solely to assist the Examiner, Applicants provide a figure from Chapter 9 entitled "Liposomes and ISCOMs" of "Novel Vaccine Strategies," S.H.E. Kaufmann (*ed.*) Wiley (2004), p. 174., attached as Exhibit B, comparing a diagrammatic representations and electron micrographs of liposomes (panel A) and ISCOMs (panel D). As can be seen from panel A, liposomes do not adopt the distinctive micro-particle structure in the form of a cage-like matrix typical of ISCOMs (panel D), and as required by the currently pending claims.

Lastly, the Examiner recites points 2-6 of the Applicants' previous argument and states that none of them were found persuasive. (OA at page 3-4). Applicants believe that these allegations are rendered moot by the above arguments. As such, it is respectfully requested that the Examiner withdraw the rejection.

Rejections under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 2, 4-6, 9, 10, 13, 16-18, 20, 21, 23-27, 32-34, 38-43, and 46-61 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Foldvari and British Pharmacopoeia 1993 (herein after "BP"). (OA at page 8). Applicants respectfully traverse this rejection.

The factors to be considered under 35 U.S.C. § 103(a), are the scope and content of the prior art; the differences between the prior art and the claims at issue; and the level of ordinary skill in the pertinent art. *See Graham v. John Deere*, 86 S.Ct. 684 (1966) and MPEP §2141. This analysis has been the standard for 40 years, and remains the law today. *See KSR International Co v. Teleflex Inc.*, 127 S.Ct. 1727 (2007). The Office has recently published Examination Guidelines to aid Examiners in formulating obviousness rejections. *See Examination Guidelines for Determining Obviousness under 35 U.S.C. 103 in view of the Supreme Court decision in KSR International v. Teleflex Inc.* Fed. Reg. Vol. 72, pp. 57526 to 57535 (October 10, 2007), hereinafter "the Examination Guidelines." Seven rationales are suggested by which obviousness may be found, *e.g.*, by combining elements in the art or substituting one known element for another. As a common thread through all the rationales, the Examiner must establish on the record that a person of ordinary skill in the art would have recognized that the results of the combination or substitution were *predictable*. *Id., e.g.*, at 57529.

The Examiner has not met the burden of establishing a *prima facie* case of obviousness based on the Examination Guidelines. Specifically, the Examiner has not established that the ordinary artisan reading Foldvari and BP in combination would arrive at the presently claimed construct for transdermal delivery of a complex

comprising a saponin and a ***cationic sterol*** which adopts a ***micro-particle structure*** in the form of a ***cage-like matrix***. Foldvari is directed to ***flexible*** lipid bilayer structures which ***fully enclose*** an oil in water emulsion. Nothing in Foldvari would even vaguely lead the skilled person to consider the use of rigid microparticles in the form of a cage-like matrix. Indeed, even the unskilled person would understand that an oil in water emulsion could simply not be contained by a microparticle with a cage-like matrix. Furthermore, the Examiner stated that Foldvari discloses sterols such as cholesterol, coprostanol, cholestanol, and cholestane, and that "absent evidence to the contrary, the sterol of Foldvari is necessarily a cationic sterol." (OA at p. 10). As discussed above, sterols disclosed in Foldvari are not cationic sterols, because none of them carry a net positive charge at pH 7.0.

With regards to the BP reference, the Examiner stated that it teaches wound dressings and medicated bandages, which include a semipermeable hydrocolloid dressing. (OA at p. 15). Furthermore, the Examiner argued that "it would have been obvious at the time the invention was made to use hydrocolloid dressing of British Pharmacopoeia 1993 because it is a sterile, self-adhesive, waterproof, multi-component structure that would be effective in delivering at least one immunogen to an individual." *Id.* Nothing in the BP reference cures the shortcomings of Foldvari as discussed above, nor would the BP reference predictably lead a skilled person to the elements missing from Foldvari such as the requirement for an immunogen delivery system comprising complex, which assumes a microparticle structure in the form of a cage-like matrix, and which comprises a cationic sterol. Therefore, the art cited by the Examiner would not have provided a reasonable expectation of success in obtaining the presently claimed

cage-like complexes comprising cationic sterols because the mere substitution or combination of elements from the two references cited by the Examiner would not have predictably resulted in a construct for transdermal delivery of immunogenic agents comprising a complex formed of a saponin and a cationic sterol, which adopts a micro-particle structure in the form of a cage-like matrix.

With respect to points 2-5 of the Applicants' previous arguments recited by the Examiner on pp. 9-16 of the OA, Applicants believe that the foregoing argument render the Examiner's contentions moot. Therefore, Applicant respectfully requests withdrawal of the rejection as it relates to the currently pending claims.

The Examiner further rejected claims 1, 2, 4, 5, 7-9, 10, 13, 16-27, 32-34, 38-43, and 46, 47, 49-55, and 57-61 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Foldvari and Lee *et al.*, *Intl. J. Pharm.* (2001) 221:1-22 (hereinafter as "Lee"). Applicants respectfully traverse this rejection.

Applicants respectfully contend that Examiner has not established that the ordinary artisan reading Foldvari and Lee in combination would arrive at the presently claimed construct. The Examiner stated that Foldvari discloses sterols such as cholesterol, coprostanol, cholestanol, and cholestane, and that "absent evidence to the contrary, the sterol of Foldvari is necessarily a cationic sterol." (OA at p. 17). As discussed above, sterols disclosed in Foldvari are not cationic sterols, because none of them carry a net positive charge at pH 7.0. The Lee reference discloses that hydrogels have been widely used as a drug carries. According to the Examiner:

> It would have been obvious at the time the invention was made to use hydrogel because of its ease in manufacturing and self application (see Lee et al. at Page 10). Moreover, it would have been obvious at the time the invention was made

> to use the cross-linked hydrogel because all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention.

(OA at p. 21).

Just as with the BP reference, nothing in the Lee reference cures the shortcomings of Foldvari as discussed above, nor would the Lee reference predictably lead a skilled person to the elements missing from Foldvari such as the requirement for an immunogen delivery system comprising complex, which assumes a microparticle structure in the form of a cage-like matrix, and which comprises a cationic sterol. Therefore, for the reasons discussed above, the combinations of references cited by the Examiner would not have predictably led the skilled artisan to the presently claimed cage-like complexes comprising cationic sterols just by performing the mere substitution or combination of elements from the two references.

With respect to points 2 and 3 of the Applicants' previous arguments recited by the Examiner on pp. 17-21 of the OA, Applicants believe that the foregoing argument render the Examiner's contentions moot. Therefore, Applicants respectfully request withdrawal of the rejection as it relates to the currently pending claims.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 23, 56, 58, and 59 under 35 U.S.C. § 112, first paragraph, as "containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time application was filed, had possession of the claimed invention." (OA at p. 22).

Specifically, the Examiner stated that the phrase "an immunologically cross-reactive antigen" added to claim 23 did not appear in the specification or original claim as filed. Applicants respectfully disagree, but in an effort to advance prosecution, and not in acquiescence to the Examiner's rejection, Applicants have amended claim 23 to delete this phrase. Additionally, the Examiner stated that the phrase "microparticles with an average diameter of not more than 50 nm" added to claim 56 did not appear in the specification or original claim as filed. Again Applicants disagree, but in an effort to advance prosecution, and not in acquiescence to the Examiner's rejection, Applicants have amended claim 56 to substitute this phrase with "about 5 nm to 50 nm." Support for this amendment can be found in paragraph [0013] of the original specification, as filed. Lastly, the Examiner argued that phrases "rigid microparticles" and "case-like structures" added to claims 58 and 59, respectively, constituted new matter. Cancellation of claims 58 and 59 render this rejection moot. Furthermore, Applicants respectfully point out that the amendment to claim 1, in which the immunogen delivery system complex "adopts a micro-particle structure in the form of a cage-like matrix" is clearly described in the present application at paragraph [0133] in a section headed "Immunogen Delivery System" notes that the complexes: "in one preferred embodiment ***adopt a micro-particle structure in the form of a cage-like matrix*** similar to that known as an immune stimulating complex (iscom)" (emphasis added). Therefore, Applicants respectfully request withdrawal of the rejection as it relates to the currently pending claims.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1, 2, 4-10, 13, 16-18, 20, 21, 23, 25-27, 32, 34, and 38-61 under 35 U.S.C. § 112, second paragraph, as "being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." (OA at p. 23). The Examiner, however, has not pointed to any specific phrases or terms which allegedly render the claims vague or indefinite. The Examiner did make some specific 35 U.S.C. § 112, second paragraph rejections of earlier-pending claims in the previous non-final Office Action mailed on October 3, 2008. In the pending Office Action at p. 2, however, the Examiner indicated that these rejections had been withdrawn. Insofar as the Examiner meant to reject claims 1, 2, 4-10, 13, 16-18, 20, 21, 23, 25-27, 32, 34, and 38-61 under 35 U.S.C. § 112, second paragraph, Applicants respectfully traverse. Should the Examiner maintain the rejection, Applicants respectfully ask the Examiner to point out any specific concerns.

Additionally, the Examiner rejected claim 59 as being vague and indefinite by the use of the term "case-like structure". Applicants respectfully submit that cancellation of claim 59 renders this rejection moot.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will

expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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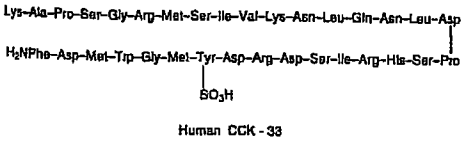
1067043_1.DOC

Am. Chem. Soc. **60**, 940 (1938). Sol in benzene, xylene, toluene. Slightly sol in methanol. Insol in water. May be solubilized by aq solns of Na desoxycholate.

Molecular complex with 2,4,7-trinitrofluorenone. Olive green crystals, mp 245-246°: Orchin, Woolfolk, *J. Am. Chem. Soc.* **68**, 1727 (1946).

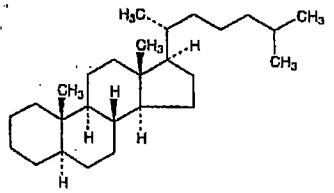
2198. Cholecystokinin. [9011-97-6] Pancreozymin; cholecystokinin-pancreozymin; CCK-PZ. Polypeptide hormone found in the mammalian gastrointestinal tract and brain. Stimulates pancreatic exocrine secretion and growth. May also play a role in appetite satiation, pain perception, and neuronal transmission. First shown to cause gallbladder contraction: Ivy, Oldberg, *Am. J. Physiol.* **86**, 599 (1928). Discovery of a substance, designated as pancreozymin, which promotes secretion of digestive enzymes by the pancreas: Harper, Raper, *J. Physiol. (London)* **102**, 115 (1943). Identity with pancreozymin: Jorpes *et al., Acta Chem. Scand.* **18**, 2408 (1964). The *C*-terminal pentapeptide has been shown to be identical to that of gastrin and caerulein: V. Mutt, J. E. Jorpes, *Eur. J. Biochem.* **6**, 156 (1968); *eidem, Biochem. J.* **125**, 57P (1971). Various biologically active, amino-truncated forms have been identified. Cholecystokinin consisting of 33 amino acids (CCK-33) is the predominant gastrointestinal form; CCK-39 and CCK-58 have also been identified. CCK-8 is the predominant CNS form. Identification of CCK in brain: J. J. Vanderhaeghen *et al., Nature* **257**, 604 (1975); G. J. Dockray, *ibid.* **264**, 568 (1976). Distribution and molecular heterogeneity: J. F. Rehfeld, *J. Biol. Chem.* **253**, 4022 (1978). Synthesis of the *C*-terminal dodecapeptide: M. A. Ondetti *et al., J. Am. Chem. Soc.* **92**, 195 (1970). Synthesis of the *N*-terminal hexapeptide of porcine CCK-33: Bodanszky *et al., J. Org. Chem.* **37**, 2303 (1972). Cloning and nucleotide sequence of the human cholecystokinin gene: Y. Takahashi *et al., Proc. Natl. Acad. Sci. USA* **82**, 1931 (1985). Total synthesis of porcine CCK-33: Y. Kurano, *Chem. Commun.* **1987**, 323; of human CCK-33: N. Fujii *et al., ibid.* **1988**, 324. Proposed role in suppression of food intake: M. A. Della-Fera, C. A. Baile, *Science* **206**, 471 (1979); C. J. Savory, M. J. Gentle, *Experientia* **36**, 1191 (1980); M. A. Della-Fera *et al., Science* **212**, 687 (1981); in regulation of hypothalamic peptides: S. Itoh *et al., Life Sci.* **25**, 1725 (1979); in modulation of catecholaminergic activity: K. Fuxe *et al., Eur. J. Pharmacol.* **67**, 329 (1980). There is also evidence that CCK acts as a specific antagonist of opiate analgesia: P. L. Faris *et al., Science* **219**, 310 (1983). *Reviews:* E. Straus, R. S. Yalow, *Fed. Proc.* **38**, 2320-2324 (1979); V. Mutt, *Biochem. Soc. Trans.* **8**, 11-14 (1980); *idem, Vitam. Horm.* **39**, 231-426 (1982). Review of physiology: G. J. Dockray, *Br. Med. Bull.* **38**, 253-258 (1982); of role in appetite satiation and pain perception: G. Stacher, *Psychoneuroendocrinology* **11**, 39-48 (1986). Symposium on neuronal CCK: *Ann. N.Y. Acad. Sci.* **448**, 1-697 (1985).



Human CCK - 33

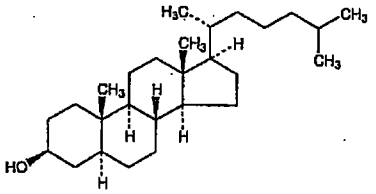
C-Terminal octapeptide *see* Sincalide.

2199. (5α)-Cholestane. [481-21-0] $C_{27}H_{48}$; mol wt 372.67. C 87.02%, H 12.98%. The *trans*-decalin homolog of coprostane, *q.v.* Prepd from cholesteryl chloride: Diels, Linn, *Ber.* **41**, 548 (1908); Windaus, *Ber.* **50**, 136 (1917); Ruzicka *et al., Helv. Chim. Acta* **16**, 327 (1933). Crystal structure: Haner, Norton, *Acta Crystallogr.* **20**, 930 (1966).



Scales from ether + alcohol, mp 80°. $[\alpha]_D^{20}$ +24.4° or +30.2° (c = 2 in chloroform). n_D^{88} 1.4887. Freely sol in chloroform, ether, benzene, slightly in abs alcohol.

2200. Cholestanol. [80-97-7] (3β,5α)-Cholestan-3-ol; Dihydrocholesterol; 3β-hydroxycholestane; β-cholestanol. $C_{27}H_{48}O$; mol wt 388.67. C 83.44%, H 12.45%, O 4.12%. Occurs in human feces, in gallstones, in eggs. Prepn by reduction of cholesterol: Willstätter, Mayer, *Ber.* **41**, 2199 (1908); Ellis, Gardner, *Biochem. J.* **12**, 72 (1918). From coprostenone: Diels, Abderhalden, *Ber.* **39**, 884 (1906). *See also* Bruce, Ralls, *Org. Synth.* coll. vol. **II**, 191 (1943).

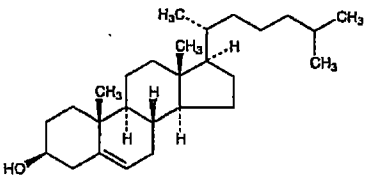


Monohydrate, scales from alc, mp 141.5-142°. $[\alpha]_D^{22}$ +24.2° (c = 1.3 in chloroform). One gram dissolves in about 100 ml alcohol, in 200 ml dry methanol. Freely sol in hot alc, ether, chloroform. Pptd by digitonin.

Methyl ether. $C_{28}H_{50}O$. Needles from acetone, mp 82.5-83°. $[\alpha]_D^{20}$ +20.0°.

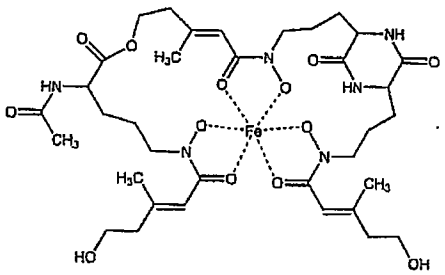
Acetate. $C_{29}H_{50}O_2$. Prisms from ethyl acetate + methanol, mp 111°. $[\alpha]_D^{20}$ +13.3° (c = 2 in chloroform).

2201. Cholesterol. [57-88-5] (3β)-Cholest-5-en-3-ol; cholesterin. $C_{27}H_{46}O$; mol wt 386.65. C 83.87%, H 11.99%, O 4.14%. Principal sterol of the higher animals. Found in all body tissues, esp in the brain, spinal cord, and in animal fats or oils. Main constituent of gallstones. Prepd commercially from the spinal cord of cattle by petr ether extraction of the nonsaponifiable matter. Also produced from wool grease. Cholesterol from animal organs always contains cholestanol (dihydrocholesterol) and other satd sterols. Purification by repeated bromination: Schoenheimer, *J. Biol. Chem.* **105**, 355 (1934); Fieser, *Org. Synth.* coll. vol. **IV**, 195 (1963). Laboratory procedure for isoln from gallstones: L. F. Fieser, *Organic Experiments* (Heath, Boston, 3rd ed., 1964) p 70. Total synthesis: Keana, Johnson, *Steroids* **4**, 457 (1964). Reviews and bibliographies: Fieser, Fieser, *Steroids* (Reinhold, New York, Chapman & Hall, London, 1959); Lettré *et al., Ueber Sterine, Gallensäuren und verwandte Naturstoffe* (Stuttgart, 2nd ed., 1955); R. P. Cook, *Cholesterol (Chemistry, Biochemistry and Pathology)* (Academic Press, New York, 1958) 542 pp; J. T. Gwynne, J. F. Strauss, *Endocr. Rev.* **3**, 299-329 (1982).



Monohydrate, pearly leaflets or plates from dil alcohol. Becomes anhydr at 70-80°. When anhydr mp 148.5°. Has been sublimed as orthorhombic needles. $bp_{0.5}$ 233°; bp_{760} 360° (some decompn). d 1.03 (monohydrate); d_{19}^{19} 1.052 (anhydr). $[\alpha]_D^{20}$ −31.5° (c = 2 in ether); $[\alpha]_D^{20}$ −39.5° (c = 2 in chloroform). Absorption spectrum: Heilbron *et al., J. Chem. Soc.* **1928**, 47. Practically insol in water (about 0.2 mg/100 ml H_2O). Slightly sol in alc (1.29% w/w at 20°), more sol in hot alc (100 g of satd 96% alcoholic soln contains 28 g at 80°). One gram dissolves in 2.8 ml ether, in 4.5 ml chloroform, in 1.5 ml pyridine. Also sol in benzene, petr ether, oils, fats. Soly in aq solns of bile salts: Rosin, *Z. Physiol. Chem.* **124**, 282 (1923). Solubilization: Gemant, *Life Sci.* **1**, 233 (June 1962). Is pptd by digitonin. Gives intense red color with rosaniline in chloroform soln.

EXHIBIT A

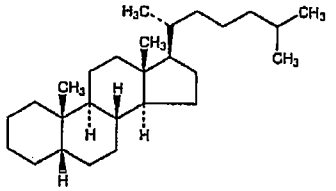


Clusters of brick-red needles from ethanol. Absorption max (ethanol): 217, 250, 440 nm (log ε 4.46, 4.22, 3.47). Sol in water, methanol, ethanol (amorphous coprogen only), propanol, benzyl alcohol, Cellosolves. Practically insol in ether, ethyl acetate, chloroform, benzene, Cellosolve esters.

Desferricoprogen. Iron-free coprogen, may be obtained by treating an aq solution with 8-quinolinol yielding a light brown powder.

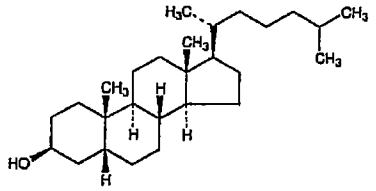
USE: Coprogen as growth-promoting agent in various organisms. Desferricoprogen to bring about excretion of iron.

2523. Coprostane. [481-20-9] 5β-Cholestane; pseudocholestane. $C_{27}H_{48}$; mol wt 372.67. C 87.02%, H 12.98%. The *cis*-decalin homolog of cholestane. Prepn: Wieland, Jacobi, *Ber.* **59**, 2064 (1926); Young *et al.*, *J. Am. Chem. Soc.* **81**, 1452 (1959); Dart, Henbest, *J. Chem. Soc.* **1960**, 3563; Nickon, Bagli, *J. Am. Chem. Soc.* **83**, 1498 (1961); Caglioti, Grasselli, *Chem. Ind. (London)* **1964**, 153.



Needles from alcohol, mp 72°. $[\alpha]_D^{11}$ +25.1° (c = 2 in $CHCl_3$). n_D^{88} 1.4884. Freely soluble in ether, chloroform; slightly in abs alcohol.

2524. Coprosterol. [360-68-9] (3β,5β)-Cholestan-3-ol; 3β-coprostanol; stercorin. $C_{27}H_{48}O$; mol wt 388.67. C 83.44%, H 12.45%, O 4.12%. Found in feces of man and of carnivorous animals: Bondzynski, Humnicki, *Z. Physiol. Chem.* **22**, 396 (1896); Wells *et al.*, *Arch. Biochem. Biophys.* **57**, 437 (1955); Samuel *et al.*, *J. Chromatogr.* **14**, 508 (1964). Prepn from coprostenone: Ruzicka *et al.*, *Helv. Chim. Acta* **17**, 1407 (1934); Shoppee, Summers, *J. Chem. Soc.* **1950**, 687. From coprostenol: Schönheimer, Evans, *J. Biol. Chem.* **114**, 567 (1936); Ruzicka *et al.*, *Helv. Chim. Acta* **21**, 498 (1938); Agashe, Summers, *J. Chem. Soc.* **1957**, 3107; Dart, Henbest, *ibid.* **1960**, 3563. Prepn from cholesterol: Rosenfeld, Gallagher, *Steroids* **4**, 515 (1964).



Needles from methanol, mp 101°. $[\alpha]_D^{18}$ +28° (c = 1.8 in $CHCl_3$). Freely sol in ether, chloroform, benzene; slightly sol in methanol (one gram dissolves in 145 ml MeOH). Insol in water.

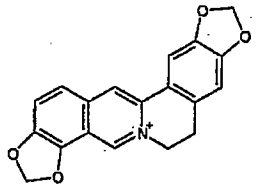
2525. Coptine. [1391-14-6] From root of *Coptis trifolia* Salisb., *Ranunculaceae:* Gross, *Am. J. Pharm.* **45**[4], 193 (1873); Mollett, Christensen, *J. Am. Pharm. Assoc.* **23**, 310 (1934); from *C. teeta* Wall.: Chatterjee *et al.*, *J. Indian Chem. Soc.* **28**, 97 (1951); from *C. chinensis* Wils.: Schramm, Tang, *Pharmazie* **14**, 405 (1959).

Crystals. Practically insol in water, ammonia, alkali hydroxides; sol in hot alcohol, dil acids.

2526. Coptis. Goldthread. Dried plant of *Coptis trifolia* Salisb., *Ranunculaceae. Habit.* U.S., northern and middle Atlantic States; Canada. *Constit.* Berberine, coptine.

THERAP CAT: Bitter tonic.

2527. Coptisine. [3486-66-6] 6,7-Dihydrobis[1,3]benzodioxolo[5,6-*a*:4′,5′-*g*]quinolizinium; 7,8,13,13a-tetradehydro-2,3:9,10-bismethylenedioxyberbinium; bis[methylenedioxy]protoberberine. $[C_{19}H_{14}NO_4]^+$. From root of *Coptis japonica* Makino, *Ranunculaceae.* Extraction procedure: Kitasato, *C.A.* **21**, 2700 (1927). Structure: Späth, Posega, *Ber.* **62**, 1029 (1929); *cf.* Huang-Minlon, *Ber.* **69**, 1744 (1936). Prepn from rhoeagenine diol: Klásek *et al.*, *Tetrahedron Lett.* **1968**, 4549.



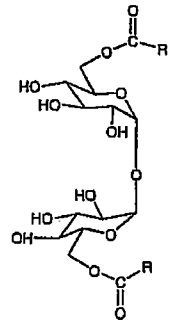
Hydroxide. $C_{19}H_{15}NO_5$. Yellowish needles from alc, mp 218°. Absorption spectrum: Kitasato, *Acta Phytochim.* **3**, 175 (1927). Very slightly sol in water; sparingly in alcohol; sol in alkalies.

Chloride. Orange prisms, not melted at 300°.

Iodide. Yellow needles, dec above 280°.

Sulfate. Yellow crystals, insol in water and alcohol (enables separation from berberine and worenine).

2528. Cord Factor(s). [61512-20-7] Trehalose 6,6′-dimycolate; 6,6′-di-*O*-mycolyl-α,α-trehalose; (6-*O*-mycolyl-α-D-glucopyranosyl)-6-*O*-mycolyl-α-D-glucopyranoside. Naturally occurring 6,6′-diesters of trehalose and mycolic acids, β-hydroxy-α-branched long chain acids produced by *Mycobacteria, Nocardia* and *Corynebacteria.* 3 major classes identified which are based on the size of the acid and the producing strain: corynomycolic acids (C_{32}), nocardomycolic acids (C_{48}-C_{60}); mycobacteria mycolic acids (C_{60}-C_{90}). The formation of "cords" by tubercule bacilli was first described in 1884 by R. Koch. Isolation of the factor from *Mycobacterium tuberculosis:* H. Bloch, *J. Exp. Med.* **91**, 197 (1950). Structure: J. Asselineau, E. Lederer, *Biochim. Biophys. Acta* **20**, 161 (1955). Synthesis: J. Polonsky *et al.*, *Carbohydr. Res.* **65**, 1 (1978); Y. Gama, *Yakugaku* **44**, 671 (1995). Antitumor activity: Bekierkunst *et al.*, *Science* **174**, 1240 (1971); M. V. Pimm *et al.*, *J. Cancer* **24**, 780 (1979). Molecular packing and surface properties: R. Almog, C. A. Mannella, *Biophys. J.* **71**, 3311 (1996). Use as antigen in serodiagnosis of *Mycobacterium avium-intracellulare* complex: K. Enomoto *et al.*, *Microbiol. Immunol.* **42**, 689 (1998); in tuberculosis: J. Pan *et al.*, *ibid.* **43**, 863 (1999). Review of the literature: E. Lederer, *Springer Semin. Immunopathol.* **2**, 133 (1979). Review of classes of cord factors and their chemistry: *idem, Microbiol. Ser.* **15**, 361-378 (1984).



R = Mycolic acids

EXHIBIT B

Novel Vaccination Strategies

Edited by
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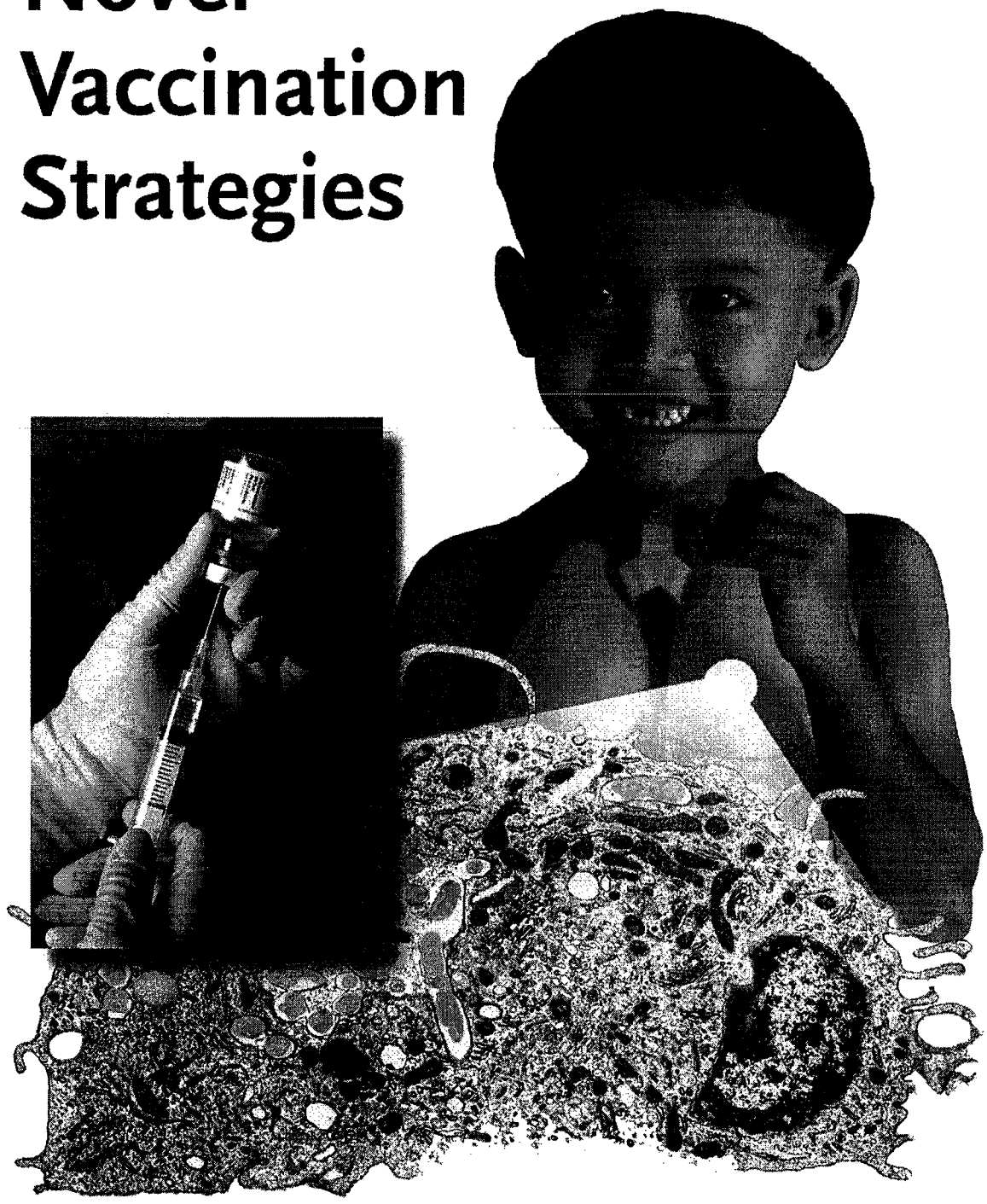

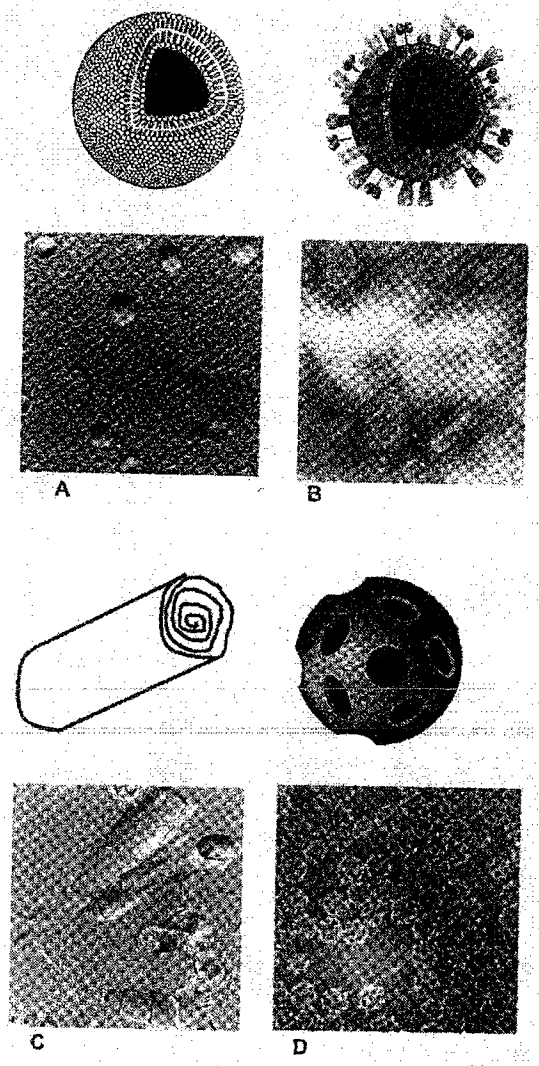

Fig. 9.1 Diagrams and electron micrographs of liposomes (A), virosomes (B), cochleates (C), and ISCOMs (D). Liposomes shown are small unilamellar vesicles (about 100 nm) prepared by detergent removal. The virosome size is about 150 nm. The virosome diagram shows hemagglutinin (trimers) and neuraminidase. Cochleates are fused bilayers kept rolled up by intercalated calcium ions. ISCOMs are profoundly different from lipid vesicles. Their main constituents are cholesterol and saponin from *Quillaja saponaria*. Size is 40 nm. Micrographs A and C are freeze-fracture images. B and D are negative staining. Sources: (A, diagram) W. Jiskoot, University of Utrecht, (A, micrograph) A. Verkleij, University of Utrecht, (B, diagram) T. Wyler, University of Berne. Reprinted from *Vaccine 21*, Zurbriggen, 921, ©2003 Elsevier Science, with permission, (B, micrograph) Waeltie and Gluck, *Int. J. Cancer 77*, 728, ©1998 Wiley-Liss. Used by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. (C, micrograph) L. Zarif et al., *Drug Delivery 2*, **2002**, with permission, (D, diagram) Reprinted from [5] ©1995 Elsevier Science with permission, (D, micrograph) courtesy of K. Teppema, M. Burger, RIVM, Bilthoven.

some may disagree on the classification 'liposome-like' for some vehicles, e.g., niosomes, cochleates, or outer membrane vesicles. Sometimes a different name for a certain vesicle type is merely semantic or guided by commercial motives, and sometimes the structures are really different from classical liposomes. Unlike ISCOMs, most liposomal structures contain an internal space separated from the environment (except for cochleates, Figure 9.1C). This allows the incorporation of hydrophilic antigens (or adjuvants), apart from bilayer association of more amphiphilic antigens, although it is difficult to achieve high loading yields. ISCOMs, on the other hand, are spherical structures, typically 40 nm in diameter. Unlike liposomes, ISCOMs cannot be loaded with water-soluble antigens because the vesicle contains pores [9].